

# Map Location of the *Rpp1* Locus That Confers Resistance to Soybean Rust in Soybean

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## ABSTRACT

Soybean rust (SBR), caused by *Phakopsora pachyrhizi*, was first discovered in North America in 2004 and has the potential to become a major soybean [*Glycine max* (L.) Merr.] disease in the USA. Currently, four SBR resistance genes have been identified but not mapped on the soybean genetic linkage map. One of these resistance genes is the *Rpp1* gene, which is present in the soybean accession PI 200492. The availability of molecular markers associated with *Rpp1* will permit marker-assisted selection and expedite the incorporation of this gene into U.S. cultivars. We compared simple sequence repeat (SSR) markers between 'Williams 82' and the BC<sub>5</sub> Williams 82 isoline L85-2378, which contains the *Rpp1* resistance allele from the soybean accession PI 200492, for candidate regions that might contain *Rpp1*. One candidate region was found with the SSR marker BARC\_Sct\_187 on linkage group G. A population of BC<sub>6</sub>F<sub>2:3</sub> lines segregating for the *Rpp1* resistance locus was genotyped in this region on linkage group G followed by inoculation with the *P. pachyrhizi* isolate India 73-1 in the USDA-ARS Biosafety Level 3 Plant Pathogen Containment Facility at Ft. Detrick, MD. The *Rpp1* gene was mapped between SSR markers BARC\_Sct\_187 and BARC\_Sat\_064 on linkage group G.

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**Abbreviations:** cM, centimorgan; SBR, soybean rust; SSR, simple sequence repeat.

**S**OYBEAN RUST (SBR), which is caused by the pathogen *Phakopsora pachyrhizi* Syd., has recently been identified in North America (Schneider et al., 2005). It has the potential for significant yield losses and major economic damage to U.S. soybean [*Glycine max* (L.) Merr.] production (Grau et al., 2004). Weather conditions conducive to high soybean yields are also ideal for spread of SBR, which can cause yield losses up to 80% (Miles et al., 2003). Most cultivars grown in the USA are highly susceptible to SBR, leading to possible epidemics in the future if weather conditions are conducive to disease development (Miles et al., 2006).

Little information is available to soybean breeders on SBR host resistant genes for integration into modern breeding lines. Initial studies have identified four unlinked dominant resistance genes. The soybean accession PI 200492 has been described as having a single dominant gene for resistance to SBR (McLean and Byth, 1980). The locus was later named *Rpp1*, which confers an immune response (no lesions) when inoculated with certain *P. pachyrhizi* isolates, including the isolate India 73-1 (Hartwig and Bromfield, 1983). The other three resistant genes (*Rpp2*, *Rpp3*, and *Rpp4*) confer a resistant

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reaction when inoculated with certain *P. pachyrhizi* isolates, which is characterized by dark, reddish-brown lesions with few or no spores (Hartwig, 1986; Hartwig and Bromfield, 1983). The susceptible reaction phenotype to infection by *P. pachyrhizi* is characterized by distinct tan lesions with prolific sporulation (Bromfield and Hartwig, 1980). These resistance genes have been shown to be susceptible to specific isolates of *P. pachyrhizi*, and it is unknown how they will react with current isolates within the USA. Although specific *P. pachyrhizi* strains are virulent on these single gene resistant sources, it may be beneficial to pyramid these four known resistant genes into modern cultivars to create broad spectrum resistance to SBR in the USA. (Hartman et al., 2005).

One of the difficulties of integrating these resistance genes into modern cultivars is that SBR is still considered an invasive pathogen in most of the USA. Currently, all research in the USA with the foreign isolates of *P. pachyrhizi* that define the current SBR resistant genes must be done under Biosafety Level 3 (BSL-3) containment. Molecular markers have been successfully applied in crops for identifying the location of disease resistance loci and for marker-assisted selection (Concibido et al., 2004; Orf et al., 2004). Simple sequence repeat (SSR) markers, one common marker used in marker-assisted selection, are polymerase chain reaction (PCR)-based, are often highly polymorphic, and can be assayed on inexpensive gel electrophoresis systems. Soybean currently has an SSR map that contains 1019 SSR markers distributed across 20 linkage groups (Song et al., 2004). These markers can be used to identify the genome location of SBR resistant genes and to help quickly integrate these genes into modern breeding lines through marker-assisted selection. Our objective was to map the *Rpp1* resistance gene to a genetic map location to help facilitate marker-assisted selection.

## MATERIALS AND METHODS

### Plant Material

A population of 126 BC<sub>6</sub>F<sub>2</sub> lines segregating for the SBR *Rpp1* resistance allele was used in the study. L85-2378, the BC<sub>5</sub> rust resistant isoline of 'Williams 82' developed with rust resistance (*Rpp1*) from the donor parent PI 200492, was backcrossed to Williams 82 in the greenhouse in the winter of 2002–2003. L85-2378 was selected using the *P. pachyrhizi* isolate India 73-1 during each cycle of backcrossing. The BC<sub>6</sub>F<sub>1</sub> plants were grown at Urbana, IL, in 2003, and the BC<sub>6</sub>F<sub>2</sub> population was planted in a winter nursery in Puerto Rico in fall 2003. Single BC<sub>6</sub>F<sub>2</sub> plants were harvested in spring 2004 to create the BC<sub>6</sub>F<sub>2</sub> lines used in this research. Seeds of Williams 82, PI 200492, and L85-2378 used to produce plants for crossing and DNA extraction were obtained from the USDA Soybean Germplasm Collection (USDA-ARS, Univ. of Illinois, Urbana, IL).

### Rust Inoculation and Phenotyping

All SBR phenotyping was performed in the USDA-ARS FDWSRU BSL-3 Plant Pathogen Containment Facility at Ft.

Detrick, MD (Melching et al., 1983). There were two replications of the phenotyping of the *Rpp1* population due to initial space limitations in the containment facility for the first replication, which was a pilot study. The first replication consisted of 83 BC<sub>6</sub>F<sub>2</sub> lines with three BC<sub>6</sub>F<sub>3</sub> plants per line. The second replication consisted of additional BC<sub>6</sub>F<sub>3</sub> progeny of the same 83 BC<sub>6</sub>F<sub>2</sub> lines plus BC<sub>6</sub>F<sub>3</sub> progeny of 43 additional BC<sub>6</sub>F<sub>2</sub> lines. In the second replication, 10 BC<sub>6</sub>F<sub>3</sub> progeny were grown per BC<sub>6</sub>F<sub>2</sub> line. Two seeds were planted in Sunshine LC<sub>1</sub> mix (Sun Grow Horticulture Products, Bellevue, WA) per cell in a (27 × 52 cm) flat that contained 6 × 12 cells. Plants were thinned to a single plant per cell 10 d after planting. To ensure uniformity of inoculation conditions, the flats positioned on the outside edges of the greenhouse had extra border rows of a susceptible soybean cultivar (i.e., Williams 82 or Maverick). Resistant and susceptible checks were planted randomly throughout the flats and included the original donor parent of the *Rpp1* resistance allele, PI 200492, as well as the resistant isoline parent, L85-2378, and the susceptible parent, Williams 82.

Inoculations were done on 15-d-old seedlings in sets of 10 to 22 flats each. Plants were inoculated with the *P. pachyrhizi* isolate India 73-1, which has been well characterized for eliciting an immune reaction on the accession PI 200492. Inoculum was produced from urediniospores stored in liquid nitrogen that were heat shocked at 40°C for 5 min, hydrated overnight in a small plastic weigh boat above water in an enclosed Petri plate. Urediniospores were suspended in distilled water containing 0.01% Tween-20, mixed, and filtered through a 53-mm nylon screen to remove any debris or clumps of urediniospores. Urediniospores were quantified using a hemocytometer to a final concentration of 20 000 per mL, and inoculations were done using 80 mL per flat, applied with an atomizer at 20 pound-force per square inch. Immediately after inoculation, plants were placed in a dew chamber at 20° to 22°C overnight, then placed on a greenhouse bench where temperatures were maintained between 20° and 25°C. Supplemental lighting was provided by 1000-W Metalarc lights (Sylvania, Daners, MA) spaced 0.6 m apart and 1.2 m above the bench. Seventeen days after inoculation, the unifoliolate and trifoliolate leaves of each plant were evaluated for reaction to soybean rust. Resistant reactions (immune) were recorded when no lesions were observed on the unifoliolate or trifoliolate leaves (Hartwig and Bromfield, 1983). A susceptible reaction (tan) was recorded when distinct tan lesions with prolific sporulation was observed on the unifoliolate or trifoliolate leaves (Bromfield and Hartwig, 1980).

### SSR Screening for Candidate Regions

Ten seeds each of PI 200492, L85-2378, and Williams 82 were grown, and leaf tissue from the 10 plants was bulked and used for DNA extraction using the modified procedure outlined by Dellaporta et al. (1983). A total of 400 SSR markers from the integrated molecular genetic linkage map of soybean (Song et al., 2004) spaced at an average of 5-centimorgan (cM) throughout the 20 chromosomes was tested on PI 200492, L85-2378, and Williams 82. Simple sequence repeat genotyping and allele size determination were performed as described by Cregan et al. (1999). Polymorphic markers between Williams 82 and L85-2378 where L85-2378 shared an allele with PI 200492 were

considered candidate markers for screening the L85-2378 × Williams 82 population segregating for *Rpp1* resistance.

### Population SSR Screening

Before inoculation with SBR, a single leaflet was collected from the first trifoliolate or, in some instances, the whole second trifoliolate from each BC<sub>6</sub>F<sub>3</sub> plant in the two replication population screening already described. Leaf tissue was immediately frozen on dry ice. DNA was isolated from the leaf tissue using the Sigma REExtract-N-Amp Plant PCR Kit (Sigma-Aldrich, St. Louis, MO), following the manufacturer's instructions. Simple sequence repeat markers in the candidate intervals were used to genotype one BC<sub>6</sub>F<sub>3</sub> plant from the first 10 *Rpp1* BC<sub>6</sub>F<sub>2</sub> lines to confirm the SSR locus was segregating in the population. Once a marker was determined to be segregating, it was used to screen between 6 and 13 BC<sub>6</sub>F<sub>3</sub> plants from each of the 126 BC<sub>6</sub>F<sub>2</sub> lines. Simple sequence repeat genotyping was performed as described by Cregan et al. (1999), and SSR allele size differences were determined as described by Wang et al. (2003) or with a 2% agarose gel. The genotype of each F<sub>2</sub> plant was inferred from the genotypes of its F<sub>2,3</sub> progeny. Map Manager QTX v. b20 (Manly et al., 2001) was used with Kosambi's mapping function to estimate genetic distances between SSR markers and *Rpp1* in the 126 BC<sub>6</sub>F<sub>2</sub> lines of Williams 82 × PI 200492.

### RESULTS AND DISCUSSION

The initial comparison of L85-2378, PI 200492, and Williams 82 identified one genomic region where L85-2378 shared an allele with PI 200492 and was polymorphic with Williams 82. This region was identified with the SSR marker BARC\_Sct\_187 on linkage group G. Since L85-2378 resulted from five backcrosses of PI 200492 to Williams 82, the genome of L85-2378 should be on average 98.4% identical to Williams 82. This would make any remaining donor segments candidate regions that may contain the *Rpp1* gene. The candidate region was first tested on 10 plants from the population to confirm it was segregating in the population before being tested on all 126 families. The initial test confirmed that Sct\_187 did segregate in the population.

The number of resistant-to-susceptible lines in the population fit the expected 3:1 ratio (Table 1). A 1:2:1 segregation ratio of the single dominant resistant gene (*Rpp1*) was confirmed when the reactions of individual plants from the BC<sub>6</sub>F<sub>2,3</sub> lines were analyzed to permit the inference of BC<sub>6</sub>F<sub>2</sub> genotype. The SSR marker Sct\_187 also fit a 1:2:1 ratio in the BC<sub>6</sub>F<sub>2</sub> pop-

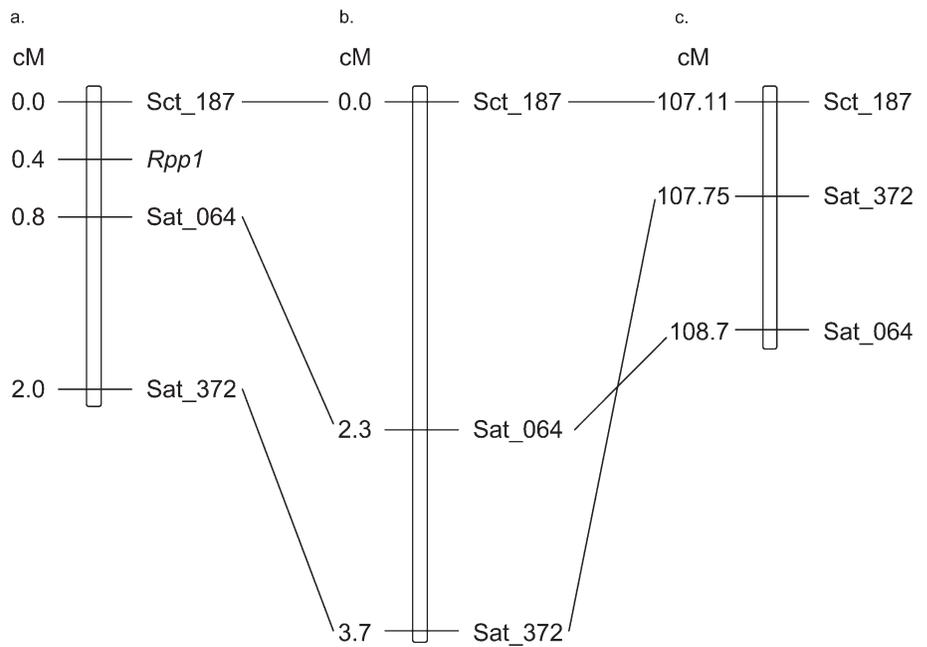


Figure 1. Genetic linkage maps of the *Rpp1* region of soybean linkage group G. The *Rpp1* resistance allele confers an immune response to the *Phakopsora pachyrhizi* isolate India-73. a. Genetic map generated using the Kosambi's mapping function from 126 BC<sub>6</sub>F<sub>2</sub> lines of Williams 82 × PI 200492. The cM values to the left of the map are cumulative distances. b. Genetic map with cumulative cM distances generated using the Kosambi's mapping function from 233 recombinant inbred lines of 'Minsoy' × 'Archer' (Song et al., 2004). c. The soybean consensus genetic map of the *Rpp1* region on linkage group G with the cumulative cM distances as reported by Song et al. (2004).

ulation as inferred from the genotypes of the BC<sub>6</sub>F<sub>2,3</sub> lines. The test for independent assortment between the *Rpp1* gene and Sct\_187 was highly significant, which indicated that the two loci are tightly linked (Table 1). Additional SSR markers were tested in the Sct\_187 region. BARC\_Sat\_372 and BARC\_Sat\_064 were also found to be polymorphic in this population, while BARC\_Sat\_117 was monomorphic. The linkage map created with the three SSR markers and the phenotypic data based on the 126 BC<sub>6</sub>F<sub>2</sub> families indicated that the *Rpp1* gene is located between Sct\_187 and Sat\_064 at a distance of 0.4 cM from each (Fig. 1). The map created from L85-2378 × Williams 82 differs from the Song et al. (2004) consensus map with an inversion at Sat\_372 and Sat\_064. The Song et al. (2004) map is based on a JoinMap analysis (Plant Research International, Wageningen, the Netherlands;

Table 1. Inheritance of soybean rust resistance and the SSR marker BARC\_Sct\_187 and the genetic linkage between the two in a population of BC<sub>6</sub>F<sub>2</sub> lines from Williams 82 × PI 200492.

Locus	Generation analyzed	Expected ratio	Observed ratio	Chi-square	Probability
<i>Rpp1</i>	BC <sub>6</sub> F <sub>2</sub>	3:1	94:32	0.01	0.92
<i>Rpp1</i>	BC <sub>6</sub> F <sub>2,3</sub>	1:2:1	32:62:32	0.03	0.99
Sct_187	BC <sub>6</sub> F <sub>2</sub>	1:2:1	30:65:31	0.14	0.93
<i>Rpp1</i> /Sct_187	BC <sub>6</sub> F <sub>2</sub>	3:6:3:1:2:1 <sup>†</sup>	30:64:0:0:1:31	120.9	< 0.0001

<sup>†</sup>Expected ratio of independent segregation of one dominant and one codominant locus R<sub>1</sub>/AA: R<sub>1</sub>/Aa:R<sub>1</sub>/aa:rr/AA:rr/Aa:rr/aa (R<sub>1</sub> immune, rr tan lesions, AA homozygous for the PI 200492 allele, aa homozygous for the Williams 82 BARC\_Sct\_187 allele, Aa heterozygous).

Van Ooijen and Voorrips, 2001) of five mapping populations. To investigate which map order is correct, we examined the genotype data in the individual populations used to create the consensus map. One of the populations used by Song et al. (2004) was the Minsoy  $\times$  Archer recombinant inbred line population and was the only population in which all three of the markers Sct\_187, Sat\_064, and Sat\_372 were analyzed. When the order of these markers was determined in the Minsoy  $\times$  Archer population alone, the order agrees with that determined in the present study (Fig. 1). The apparent inversion in the consensus map is most likely caused by the JoinMap analysis of the multiple populations.

Sct\_187 is in a genomic region that has had association with other disease resistance loci. Concibido et al. (1997) found a minor soybean cyst nematode resistance gene linked to A378\_1, which is 2 cM away from Sct\_187. The *Rps4*, *Rps5*, and *Rps6* resistance genes to *Phytophthora* root rot (caused by *Phytophthora megasperma* Drechs. F. sp. *glycinea* Kuan and Ervin) have been mapped to this same region on LG G (Demirbas et al., 2001; Diers et al., 1992).

The tight linkage of the flanking markers Sct\_187 and Sat\_064 to *Rpp1* makes these SSR markers useful for marker-assisted selection. Sct\_187 and Sat\_064 have gene diversity estimates of 0.46 and 0.84, respectively (Cregan et al., 1999), which indicates these markers will be polymorphic in a wide range of crosses. In addition to the flanking markers, the other two SSR markers in this region, Sat\_372 and Sat\_117 (Song et al., 2004), may be useful for marker-assisted selection depending on the parents of the germplasm being analyzed. Since there are only a few SSR markers available, the development of additional SSR and single nucleotide polymorphism markers will help breeders to integrate the *Rpp1* resistance allele into modern cultivars and facilitate the fine mapping of the gene.

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